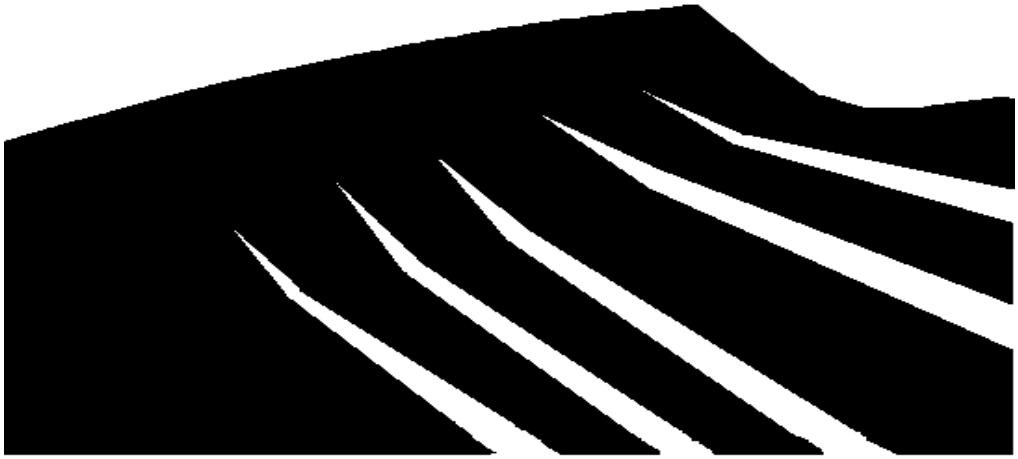
USE OF A SPECTROPHOTOMETER OR FLUOROMETER TO DETERMINE CONSTITUENT CONCENTRATIONS IN SOLUTION

LOS ALAMOS
QUALITY PROGRAM



APPROVAL FOR RELEASE

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Los Alamos
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HISTORY OF REVISION

REVISION NO.	EFFECTIVE DATE	PAGES REVISED	REASON FOR CHANGE
R0	01/18/96	N/A	Initial procedure.
R1	01/21/97	3, 4, 5, 7, 8, 9, 11, & 12	Minor non-substantive editorial changes

USE OF A SPECTROPHOTOMETER OR FLUOROMETER TO DETERMINE CONSTITUENT CONCENTRATIONS IN SOLUTION

1.0 PURPOSE

This detailed procedure (DP) describes the use of a spectrophotometer or fluorometer to determine concentrations of constituents in solution. This DP is intended to support the C-Wells Reactive Tracer Studies for the Yucca Mountain Project (YMP), although it could also be used for other YMP studies.

2.0 SCOPE

This DP applies to any procedure that involves the use of a spectrophotometer of fluorometer to determine concentrations of constituents in solution. It is beyond the scope of this DP to describe all possible concentration measurement procedures involving a spectrophotometer or fluorometer, so the user must document the method used and the results obtained for each application. This DP does not apply to concentration measurement procedures where a spectrophotometer or fluorometer is used only as a minor component (e.g., when spectrophotometer of fluorometry is used to determine the end point of a titration). In these cases, the process should be documented in a field or laboratory notebook in accordance with QP-03.5, or it should be incorporated into another DP.

3.0 REFERENCES

LANL-EES-4-DP-802, Preparation of Standards for Tracer Concentration Measurements.
LANL-YMP-QP-02.7, Personnel Training
LANL-YMP-QP-03.5, Documenting Scientific Investigations
LANL-YMP-QP-08.1, Identification and Control of Samples
LANL-YMP-QP-17.6, Records Management
Mann, C.K., Vickers, T.J., and Gulick, W.M., “Instrumental` Analysis.” Harper and Row, New York (1974).

4.0 DEFINITIONS

4.1 Spectrophotometer

An instrument that is capable of focusing a beam of light of a specified wavelength or wavelength range on a sample containing a constituent that absorbs the light to some degree. The instrument often measures the absorbance of light by splitting the light beam (using optics) and comparing the amount of light transmitted through the sample to the amount of light transmitted through a sample that does not contain the light-absorbing constituent (a blank).

4.2 Fluorometer

An instrument that is capable of focusing a light beam of a specified wavelength or wavelength range on a sample containing a constituent that is excited by the light. The excited constituent then emits light at a different (longer) wavelength than the incident light; this process is called fluorescence. The intensity of the fluorescent light is typically measured at a 90° angle from the incident light beam to minimize interference from the incident beam.

5.0 RESPONSIBILITIES

The following personnel are responsible for the activities identified in Section 6.0 of this procedure.

- Principal Investigator (PI)
- Procedure User

6.0 PROCEDURE

The use of this procedure must be controlled as follows:

- If this procedure cannot be implemented as written, YMP personnel should notify appropriate supervision. If it is determined that a portion of the work cannot be accomplished as described in this DP, or would result in an undesirable situation, that portion of the work will be stopped and not resumed until this procedure is modified or replaced by a new document, or until the current work practice is documented in accordance with QP-03.5 subsection 6.1.6.

- Employees may use copies of this procedure printed from the controlled document electronic file; however, employees are responsible for assuring that the correct revision of this procedure is used.

- When this procedure becomes obsolete or superseded, it must be destroyed or marked “superseded” to ensure that this document is not used to perform work.

6.1 Principle

Spectrophotometers and fluorometers measure the amount of light either absorbed or emitted (fluoresced) at a given wavelength by a constituent in solution. The theory behind the operation of these instruments is described in detail in most analytical chemistry or instrumental analysis texts (e.g., Mann, Vickers, and Gulick, 1974). These instruments typically provide an analog or digital readout that is proportional to the concentration of the absorbing or fluorescing constituent in solution. However, the readout is not an absolute measurement, so when measuring unknown concentrations, it is common practice to prepare a series of standards of known concentration to establish a calibration curve of concentration vs. readout that can be used to determine unknown concentrations. The excitation and emission wavelengths can

generally be adjusted on the instrument to optimize the sensitivity of the measurements.

6.2 Equipment and Hardware/Software

The equipment and hardware/software associated with this DP consists of (1) a spectrophotometer or fluorometer, which typically includes a light source, optical equipment (e.g., filters) to control incident or sensed wavelengths, light detectors/photomultiplier tube(s), and electronics to control the instrument and convert the sensed light intensity to a readout, (2) special test tubes or cuvettes to contain the samples, (3) a flow-through cell (on some models) that allows continuous monitoring of absorbance or fluorescence in a flowing stream, and (4) on more sophisticated models, environmental (e.g., temperature) control equipment. Other equipment might include a computer interfaced to the instrument for control and/or data logging, and filtration equipment used to remove particulates from the samples that could interfere with the absorption or fluorescence measurements. This list of equipment is not intended to be all-inclusive.

6.2.1 Equipment Malfunctions

Malfunctions of any of the equipment described above will be either immediately obvious to the user or will result in the inability to conduct the procedures described in this DP.

6.2.2 Safety Considerations

Safety considerations will depend on the chemical nature of the solutions being analyzed. Material safety data sheets (MSDSs) should be consulted to determine whether special protective clothing and/or eye protection are required. Hazardous chemical wastes should be properly disposed of. Other than the hazards of electrical shock from standard 120 volt electrical outlets or from internal instrument electronics, there are no hazards associated with the operation of the equipment described above. A maintenance technician certified by the manufacturer or vendor should be contacted for nonroutine maintenance of all spectrophotometers or fluorometers.

6.2.3 Special Handling

Handling of all equipment associated with this DP should be done in accordance with manufacturer's or vendor's guidelines. Special handling of equipment or hardware should be considered on a case-by-case basis as the need arises. Any special handling should be documented in a field or laboratory notebook.

6.3 Preparatory Verification

The instrument should be allowed to warm up according to the manufacturer's instructions. Depending on the type of light source, it may take two hours or more for the light to stabilize to the point where consistent, stable readings are obtained. To ensure proper warm-up, a standard solution should be measured repeatedly until the readings are stable and consistent. It is also important to ensure that all sample test tubes or cuvettes are clean and free of fingerprints prior to being used, as any dirt or oil could cause errors or inconsistencies in the measurements. Other preparations should be done in accordance with the manufacturer's instruction manual.

To ensure optimum measurement sensitivity, the wavelength controls on the instrument should be adjusted until the highest reading is obtained while a standard is in the measurement cell. For a spectrophotometer, this will involve adjusting the wavelength setting until the maximum absorbance is obtained. For a fluorometer, the excitation wavelength setting should be adjusted first to obtain the best response, and then the emission wavelength setting should be independently adjusted until the maximum reading is obtained. Note that the optimum wavelength settings may not correspond to exactly what the peak excitation, emission, or absorbance wavelengths are in the published literature, but they should generally be within a few percent of the published values.

6.3.1 Hold Points

(N / A)

6.3.2 Calibration

Calibration should be accomplished by conducting measurements on a series of standard solutions that span the expected range of concentrations of the unknowns. A reading should be obtained for each standard, and then these readings and the known concentrations should be used to construct a calibration curve. The curve is then used to determine the concentrations of unknowns given their readings. Some instruments have internal calibration features that will calculate a calibration curve if the concentrations of the standards are provided as they are measured.

If possible, the standards should be prepared using the same background solution as the unknowns. If low concentrations are expected for the unknowns, a reading should be obtained using a blank that consists of the unknown solution with no target constituent present. This will establish a lower detection limit, and it will also establish whether there are any interfering constituents in the solution that absorb or fluoresce at the same wavelength as the target constituent. Some instruments require that all measurements be made relative to a blank (that is, a blank is measured at the same time as the unknown, and the difference between the two signals is taken to be the reading). Calibrations should be

repeated at regular intervals to check for instrument drift. The interval between calibrations will depend on the manufacturer's recommendations as well as on the experience that the PI and/or DP users have with the instrument. The results of all calibrations and the times at which they were conducted should be recorded in a field or laboratory notebook in accordance with QP-03.5.

Preparation of standards associated with tracer experiments should be done in accordance with DP-802, Preparation of Standards for Tracer Concentration Measurements. Alternatively, standard preparation can be documented in a field or laboratory notebook.

6.3.3 Environmental Conditions

Measurements are most consistent and reproducible when samples and standards are measured at the same temperature. If all the standards and unknowns cannot be kept at a constant temperature, they should at least be allowed to experience the same temperature conditions.

If the same solution is not used for all standards and unknowns, care should be taken to ensure that the pH and ionic strength of all solutions are about the same, as these properties can affect the absorbance or fluorescence of samples. For example, some weak acids only fluoresce when they are dissociated, so changes in pH can affect the measured fluorescence even when the concentration of the acid is not changed.

NOTE: Some instruments come with temperature control features that allow consistent readings to be obtained even when sample temperatures are not constant. The user should document the use of any of these features.

6.4 Control of Samples

Samples, including standards, are to be identified and controlled in accordance with QP-08.1. All samples should be stored in such a way that evaporation of water is minimized or eliminated during storage by storing them in tightly capped bottles and/or refrigerating them. The sample container material should be chosen to avoid potential sorption of constituents to the containers walls. If sorption is unavoidable, special procedures should be followed to desorb the tracers prior to analyses (e.g., acidification). These procedures should be documented in a field or laboratory notebook. If there is any question about sorption to container walls, batch sorption experiments should be conducted (and documented in a field or laboratory notebook) using the constituents and labware in question. Samples should be stored such that the impact of storage on the analyses is minimized. For example, if a light sensitive constituent is to be analyzed (e.g., a fluorescent dye such as fluorescein), the samples should be stored in darkness or in opaque bottles. Special handling requirements for different constituents should be considered on an individual basis, and the handling and storage of all samples to be analyzed should be documented in a

field or laboratory notebook so that a sample handling history is maintained. This documentation should be done in accordance with QP-08.1.

6.5 Implementing Procedure

6.5.1 Sample Analysis

The procedure detailed here should be used for analyzing a series of individually collected grab samples. When analyzing samples in real time (using a flow-through cell in the instrument), the procedure, including calibrations, should be clearly documented by the PI or the DP user in a field or laboratory notebook.

After allowing the instrument to warm up properly, a series of standard solutions should be measured to establish a calibration curve (see subsection 6.3.2). The measurements on at least one standard should be repeated until it is clear that the readings are consistent and stable (see subsection 6.3).

Once a calibration curve has been established, the measurement of unknowns can begin. Although the specific procedure used to obtain readings will depend on the instrument (the instruction manual should be consulted), there are some steps and precautions that are common to the use of almost all spectrophotometers and fluorometers. First, only thoroughly cleaned test tubes or cuvettes should be used. Great care must be taken to not introduce dirt or fingerprints on the outside of the test tubes or cuvettes during a series of measurements, as these can block or absorb some of the incident light and thus cause errors in the measurements. To minimize the effect or dirt and fingerprints, the outside of each test tube or cuvette should be wiped with a lint-free towelette prior to being introduced to the instrument.

In most cases, because their supply is limited, test tubes or cuvettes will be used repeatedly to conduct a series of measurements. When this is done, care must be taken to ensure that there is no cross-contamination or carryover of one sample to the next. After emptying a sample out of a test tube or cuvette, the test tube/cuvette should be rinsed thoroughly with a blank solution (e.g., a solution with no absorbing/fluorescing constituent, such as deionized water). The test tube/cuvette should then be rinsed once or twice with the next solution to be analyzed to ensure that the blank solution has been removed and does not dilute the unknown. The amount of rinsing may be limited by the amount of unknown available, but at least two rinses of the test tube/cuvette should be attempted. The unknown should then be dispensed into the test tube/cuvette and a measurement can be taken. The reading itself can involve some subjective judgment, as many instruments have analog or digital readouts that are noisy and require the user to estimate an average value out of many that are continually displayed.

If the unknowns contain significant amounts of suspended particulate matter, it is generally desirable to filter the samples prior to introducing them to the test tubes or cuvettes because the particulates can scatter the incident or emitted light and cause measurement errors. This is especially a problem when the amount of suspended matter is not the same in each sample. Filtration can be accomplished by any means that does not introduce interfering contamination or cross-contamination between samples. Filtration media and labware should be chosen to avoid sorption of solutes of interest. The method and materials used should be documented in a field or laboratory notebook.

Calibrations should be repeated periodically to ensure that the instrument is not drifting over time. The time interval between calibrations should be as recommended by the manufacturer's instruction manual or based on the experience of the PI or DP user(s) with the particular instrument. The calibration data and times of each calibration should be recorded in a field or laboratory notebook. If significant drift occurs between calibrations, the PI or DP user must decide whether it is necessary to repeat the measurements made since the last calibration. In general, a drift of more than 5 to 6% is considered excessive, and serious consideration should be given to repeating measurements. The decision of whether or not to repeat measurements, and the rationale for it should be documented in a field or laboratory notebook. If sample volumes are limited, it may be best to simply try to account for drift by averaging or interpolating between two successive calibration curves that bracket the measurements. Any correction procedure such as this should be documented in a field or laboratory notebook.

Finally, it is generally desirable to estimate the precision associated with a series of measurements. This is usually best accomplished by repeating measurements on a given sample several times over the course of all measurements so that a statistical measure of precision (e.g., standard deviation) can be estimated. If sample volumes severely restrict the number of repeat measurements that can be made, then the repeat measurements should be made on a sample prepared from the standard (e.g., a dilution of one of the standards).

6.5.2 Documentation of Results

Documentation requirements will depend on the instrumentation and technique used. As general guidelines for measurements of grab sample concentrations, the following information should be documented for each set of measurements:

- The unique identifier of the instrument(s) used (e.g., manufacturer's name and model number serial number),

- All of the instrument settings for the measurements. For example, the wavelength settings, lamp settings (such as intensity, frequency, etc.), and any other settings associated with the instrument's electronic or optical equipment should be recorded,

- The identity and concentration of all standards (preparation of standards should also be documented; however, when analyzing samples from tracer experiments, standard preparation should be done in accordance with DP-802),

- All instrument readings obtained, including those for unknowns and standards (calibrations),

- The times at which calibrations were conducted,

- Environmental conditions, such as temperature, over the course of the measurements, and

- The method of calculating the concentrations of unknowns. For example, if calibration curves use used, a plot of each curve and the time of calibration should be recorded. If internal calibration features are used, the readings associated with each standard should still be recorded to provide future traceability.

When samples are analyzed in real time (as, for example, with a flow-through cell) or when significant deviations from the procedure described in subsection 6.5.1 are necessary, the PI should determine the documentation requirements prior to conducting the analysis. In general, the user should record all measurements and calibrations necessary to determine target constituent concentrations in the unknowns.

6.6 Data Acquisition and Reduction

Data acquisition may be done manually or automatically, depending on the instrument used. When reducing the data associated with analysis of grab samples, concentrations can be deduced directly from calibration curves. It is often convenient and desirable to employ a curve-fitting software package to fit a set of calibration data and then use the fit to determine the concentrations associated with unknown readings. Some instruments have internal software to do this and will actually provide a readout in direct concentration units. Whenever calibration curves are used to determine concentrations, it is important that the standards span the entire range of the unknowns (that is, the unknown concentrations should not be deducted by extrapolating a calibration curve). In cases where instrument drift occurs, the PI or DP user should determine whether measurements need to be repeated (see subsection 6.5.1). Corrections for drift can be crudely made by averaging two successive calibration curves that indicate drift (that is, the average readings associated with each standard at each time can be used to generate an "averaged"

calibration curve). Another method of correcting for drift is to try to interpolate that standard readings associated with a given unknown reading by assuming that the drift is linear with time (or sample number). For example, if a standard had readings of 90.0 and 95.0 before and after an unknown is measured, respectively, and the unknown was the 4th of 10 samples measured between the two standard readings, then the proper standard reading to assume for the measurement of the unknown would be 90.0 + (4/10) (95.0 - 90.0) = 92.0. It has been found that precision can be improved if drift is accounted for in this way. Any method of accounting for drift should be documented in a field or laboratory notebook.

The PI reviews the data and associated records and determines the acceptability of the generated data. The PI may reject measurements for any of the following reasons:

- anomalous results,
- unacceptably large standard deviations associated with repeat concentration measurements on the same sample, indicating poor measurement precision,
- operational deviations which call into question the accuracy of the results, and
- inadequate record keeping.

The identity of the rejected results and the basis for rejection are recorded in the field or laboratory notebook.

6.7 Potential Sources of Error and Uncertainty

Potential sources of error and uncertainty will depend on the instrument and technique used. When measuring concentrations in grab samples, the following sources of error and uncertainty may exist:

- not allowing enough time for the instrument to warm up and stabilize,
- dirt or fingerprints on test tubes or cuvettes, particulates in the samples,
- cross-contamination between samples as a result of not thoroughly rinsing out the test tubes or cuvettes between measurements,
- incorrectly reading the analog or digital readout of the instrument,
- poor preparation of standards used for calibration, and
- the presence of interfering constituents (i.e., constituents that give a response similar to the target constituent) in the unknowns that are not present in the calibration standards.

When samples are analyzed in real time (e.g., using a flow-through cell), an additional potential source of error is biological growth on the sample cell walls that results in absorbance or blockage of the incident light.

7.0 RECORDS

Records generated as a result of this DP are entries in field or laboratory notebooks or attachments to such notebooks. The documentation should consist of any applicable items identified in Section 6.0. Notebooks should be kept in accordance QP-17.6.

8.0 ACCEPTANCE CRITERIA

Proper completion and submittal of the records described in Section 7.0 constitutes the acceptance criteria for this procedure.

9.0 TRAINING REQUIREMENTS

Read-only training is required for this DP. Training is documented in accordance with QP-02.7.

10.0 ATTACHMENTS

(N / A)